The p18 component of the multisynthetase complex shares a protein motif with the β and γ subunits of eukaryotic elongation factor 1[1]

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Abstract In higher eukaryotes, nine aminoacyl-tRNA synthetases form a multienzyme complex also comprising the three auxiliary proteins p18, p38 and p43, of apparent molecular masses of 18, 38 and 43 kDa. The function of these proteins, invariably found associated to the synthetase components of the complex, is unknown. In order to gain a more precise view of the structural and functional organization of this complex, we cloned the cDNA encoding the p18 component. The 174-amino-acid hamster protein displays sequence homology with the NH_2-terminal moieties of the β and γ subunits of the elongation factor EF-1H, implicated in subunits interaction. The homologous polypeptide fragment of about 90 amino acids is also recovered in the NH_2-terminal extension of human valyl-tRNA synthetase, involved in its assembly with EF-1H. These results suggest that p18 contributes a template for association of the multisynthetase complex with EF-1H.

Key words: Aminoacyl-tRNA synthetase complex; Elongation factor 1; Protein motif; tRNA channeling

1. Introduction

In mammalian cells, several components of the protein synthesis machinery are specifically organized into multienzyme structures. In particular, this type of structural organization applies to macromolecules involved in the elongation cycle: aminoacyl-tRNA synthetases and elongation factors. The four subunits of the eukaryotic elongation factor 1, EF-1α, β, γ and δ, are associated in a complex comprising the G-protein homologous to the bacterial elongation factor EF-Tu, EF-1α, as well as the two GDP-GTP exchange factors, EF-1β and δ [1]. The nine aminoacyl-tRNA synthetases specific for amino acids Glu, Pro, Ile, Leu, Met, Gln, Lys, Arg and Asp are arranged in a multienzyme complex containing three other proteins as well [2]. In addition, a stable complex composed of stoichiometric amounts of EF-1H and valyl-tRNA synthetase has been isolated from various eukaryotic cells, from invertebrates to mammals [3–5]. The occurrence of supramolecular assemblies supports the idea according to which translation is a highly organized process in the cytoplasm of mammalian cells. Moreover, evidence in favor of the subcellular organization of the protein synthesis machinery within the cytoskeletal framework has been provided [6–9].

The molecular features responsible for the assembly of these macromolecules into well-defined complexes have been extensively studied. It is known that association of aminoacyl-tRNA synthetases is mediated by terminal polypeptide extensions, which are dispensable parts of these enzymes for catalysis [2]. Although association of valyl-tRNA synthetase and of EF-1α, ensuring consecutive steps of the tRNA cycle, aminoacylation and transfer of the activated tRNA to the ribosome, makes sense in light of the hypothesis of tRNA channeling during mammalian protein synthesis [10,11], the putative in vivo functional prevalence of the multisynthetase complex over free enzymes remains to be establish. In this paper, we show that one of the auxiliary component of the complex, the p18 protein, homologies with the polypeptide regions of valyl-tRNA synthetase and of the β and γ subunits of EF-1H which have been identified as subunits binding sites.

2. Materials and methods

The aminoacyl-tRNA synthetase complex from sheep liver was isolated as previously described [12]. The polypeptide components were fractionated by SDS-PAGE electrophoresis on a 10% polyacrylamide gel prepared according to Laemmli [13], and transferred onto ProBlott membranes (Applied Biosystems). Membrane pieces carrying p18 were cut and treated with trypsin essentially as described [14]. In situ protease digestion was carried out at 28°C for 18 h, at a protease: p18 ratio of 1:50. Peptides recovered after washing of the membranes were separated by reverse-phase HPLC on a C18 column. The amino acid sequence analysis was carried out by J.-P. Le Caer (Laboratoire de Physiologie Nerveuse, Gif-sur-Yvette) with a gas-phase sequencer (model 470A, Applied Biosystems).

Total RNA from sheep liver was prepared [15] and poly(A)$^+$ RNA was selected by oligo(dT)-cellulose chromatography. Following synthesis of the first strand cDNA according to standard procedures [16], PCR amplification of p18 cDNA was accomplished using p18-specific mixed oligonucleotides as 5′-terminal primers and a poly(dT) primer, 5′-GGGATCC$(T)_{20}$-3′, complementary to the poly(A) tract of mRNA as a 3′-terminal primer. PCR products were cloned into *Eco*RI-*Bam*HI digested M13mp18 DNA. Nucleotide sequence of the inserts was determined by the dideoxy nucleotide chain termination method [17] using the T7 sequencing kit from Pharmacia.

A cDNA library was constructed from poly(A)$^+$ mRNA isolated from exponentially growing CHO cells, in the Uni-ZAP XR vector by using the ZAP-cDNA synthesis kit (Stratagene). The lambda library was screened with PCR-selected cDNA-fragments described above, radiolabeled by random oligonucleotide priming. Following in vivo excision of recombinant pBluescript SK(−) phagemids by superinfection with R408 helper phage, the nucleotide sequence of the longest cDNA fragment was determined on both strands.

Computational searches were performed at the NCBI using the BLAST network service [18] or at the CITI2 using the BISANCE service [19].

3. Results

3.1. Isolation of the cDNA encoding the p18 component of the multi-synthetase complex

The p18 component of the multisynthetase complex from sheep liver was isolated following fractionation of the complex by polyacrylamide gel electrophoresis in the presence of SDS.

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The protein was transferred onto membranes and subjected to in situ cleavage by trypsin. Resulting peptides were separated by reverse-phase HPLC and their amino acid sequences were determined. Four distinct sequences were obtained (Table 1). The two longest sequences were chosen to design primers for PCR amplification of the target sheep cDNA. Because several amino acids corresponding to six or four possible codons were recovered, the level of degeneracy of the deduced oligonucleotides was reduced by using inosine at the third position of 10 codons. In addition, two successive amplification steps were performed to counterbalance the poor specificity of the ensuing primers. After amplification with primers p18-d2 (Table 1) and a poly(dT) primer complementary to the poly(A) tract of mRNA, followed by p18-d1 and poly(dT), a 500-nucleotide-long cDNA of sheep origin was recovered. This fragment was used to probe a CHO cDNA library constructed in the Uni-Zap XR vector. Ten independent clones were recovered and analyzed. A 833-bp cDNA accounted for the complete coding sequence of p18 (Fig. 1).

The cDNA sequence has a coding potential for a polypeptide of 174 amino acids, with a calculated molecular mass of 19.8 kDa, in good agreement with the apparent value of 18 kDa determined by SDS-PAGE. The sequences of the four trypsic peptides derived from the p18 protein (Table 1) are found in the protein sequence derived from the cloned cDNA, thereby unambiguously establishing its assignment to the p18 cDNA from CHO cells. The sequence of the sheep protein from Ala^{69} to the COOH-terminus, deduced from the PCR-isolated fragment, is 89% identical to the corresponding region of the hamster p18 (Fig. 1). Such a high level of amino acid conservation suggests that the p18 component of the complex contributes an essential well-defined functional role. No other protein displaying a high similarity score could be identified in the protein data libraries.

3.2. *Identification of homologies with proteins of known function*

By using the Blast network facilities [18], three blocks displaying sequence similarities with proteins registered in the data libraries could be identified. Block A, from Leu^{60} to Ile^{82}, block B, from Leu^{95} to Leu^{124}, and block C, from Tyr^{139} to His^{151}, were recovered in the NH_2-terminal moiety of the γ subunit of eukaryotic elongation factor 1, with a probability of matching by chance of 2×10^{-6}, whereas only blocks B and C were found in the β subunit of EF1-H (Fig. 2). The three sequence motifs were also detected within the NH_2-terminal polypeptide extension of human valyl-tRNA synthetase or of the multifunctional glutamyl-prolyl-tRNA synthetase from fly or human. The glutathione *S*-transferase protein family from the theta class, also exhibits the conserved blocks A, B and C of p18. Sequences similarities between those aminoacyl-tRNA synthetases and EF1-γ [20,21], and with the glutathione *S*-transferase superfamily [22] have been already identified. The crystal structure of a theta-class glutathione transferase is known [23]. The three conserved blocks identified in Fig. 2 are clustered within the α-helical COOH-terminal domain of the protein, distinct from the NH_2-terminal catalytic domain of the enzyme. No particular function has been ascribed so far to the corresponding region of the protein, made of four helical segments, which displays a conserved ternary structure in the transferase family despite a low level of sequence identity. Therefore, the presumably compact α-helical fold building the COOH-terminal moiety of p18 is likely to contribute a structural rather than a catalytic function.

4. Discussion

In mammalian cells, p18, p38 and p43 are ubiquitous non-synthetase components of the multisynthetase complex [2]. The occurrence of this multienzyme structure is restricted to the metazoan subgroup of coelomates, from arthropods to mammals. The cDNAs encoding seven of the nine synthetase components of the complex have been isolated, but none of the cDNA coding for the associated proteins p18, p38 and p43 had been cloned previously. In this study, we have reported the cloning and sequencing of a cDNA encoding the hamster p18 protein. Since we could not recover any homologous protein from the complete sequence of the genomes from the bacteria *Haemophilus influenzae* or *Mycoplasma genitalium*, or from the yeast *Saccharomyces cerevisiae*, it is quite possible that the functional role of the p18 protein in the complex is specifically related to this type of structural organization. It is noteworthy that blocks A, B and C are recovered from the yeast elongation factors γ and β subunits (EF1γSp, EF1βSp and EF1βSc in Fig. 2) but not in yeast aminoacyl-tRNA synthetases, enzymes that have never been isolated as high-molecular-weight complexes.

Noteworthy is the finding that the higher level of sequence similarity is recovered with another component of the translation machinery, the γ subunit of elongation factor 1. Several pieces of evidence suggest that the p18 component of the complex could contribute an anchorage for the elongation factor EF-1H. The heavy form of EF-1 is a pentameric com-

Table 1
Mixed oligonucleotide primers deduced from the amino acid sequence of the tryptic peptides of sheep p18

Peptide and oligonucleotide sequences		
Name		Sequence
pep18-1		Tyr Ser Ala Gln Gly Glu *Asn*
pep18-2		Leu Tyr Thr Asn Ser
pep18-4		Glu Tyr Leu Leu Gly Ser Thr Ala Glu
p18-d2	ggaattc	**GAR TAY YTI YTI GGI WSI ACI GCI GA**
pep18-6		Ala Val Val Gln Gln *Trp* Leu Glu Tyr *Arg*
p18-d1	ggaattc	**GCI GTI GTI CAR CAR TGG YTI GAR TA**

Amino acid sequences of tryptic fragments from the p18 component of the sheep liver complex, determined in this study, pep18-*x*, are indicated. Uncertain amino acids are italicized. Two mixed sense primers, p18-d1 and d2, 5′- to 3′-extremity, were synthesized. For codon degeneracy, abbreviations are: Y for T or C; R for A or G; W for T or A; S for C or G; I for inosine. Nucleotides introduced to generate an *Eco*RI site at the 5′-end of the primers are shown in lower case.

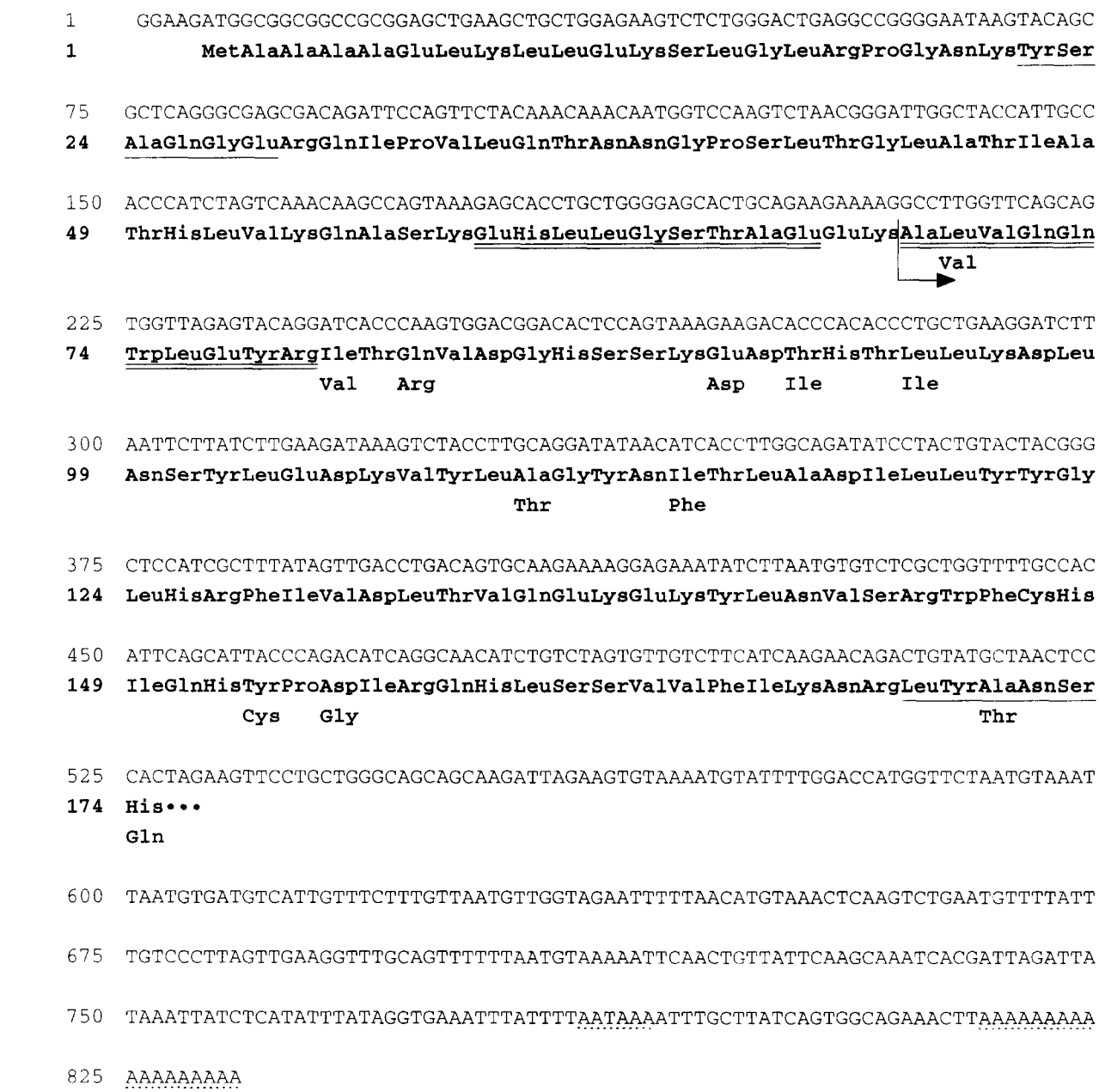
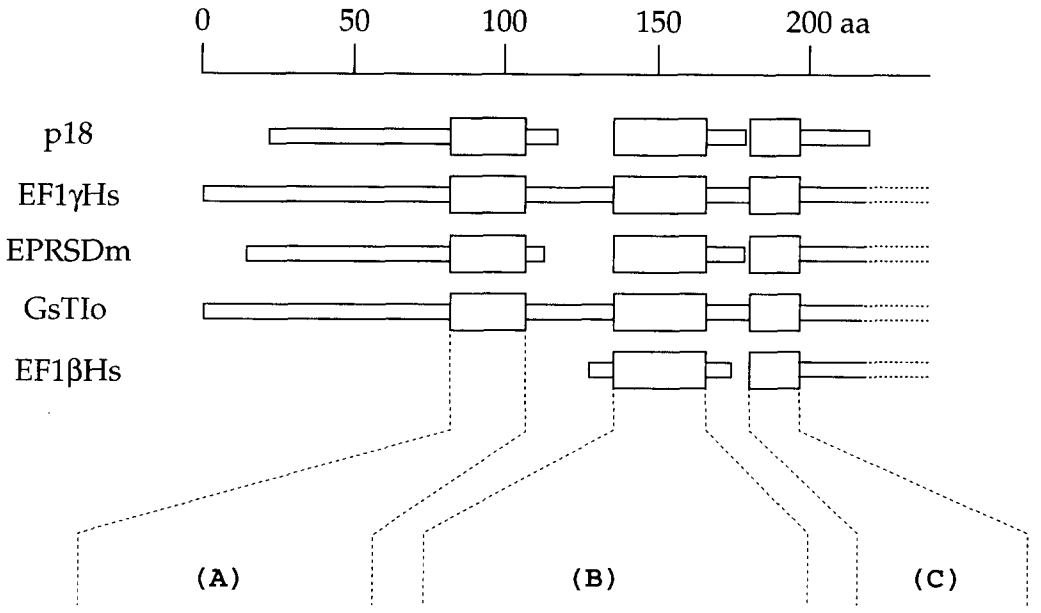


Fig. 1. Nucleotide and deduced amino acid sequence of cDNA encoding the p18 component of the hamster complex. The sequences of the four peptides shown in Table 1, determined for the sheep p18 protein, are underlined; those that were used to design oligonucleotide primers are double underlined. The putative polyadenylation signal and the poly(A) tract are indicated by a dotted line. The COOH-terminal sequence of the sheep p18 protein, deduced from the PCR fragments, starting from Ala^{69} (marked by an arrow), is indicated under the amino acid sequence of the hamster protein. Only positions where the two sequences differ are shown.

plex composed of the four subunits α, β, γ and δ in a molar ratio 2:1:1:1 [24]. EF-1α forms a ternary complex with aminoacyl-tRNA and GTP, and is the donor of aminoacyl-tRNA for ribosomal protein synthesis. After release of the tRNA and hydrolysis of GTP, activation of EF-1α·GDP to EF-1α·GTP requires the guanine nucleotide exchange factors EF-1β and EF-1δ. The role of the γ subunit is unclear. It stimulates the activity of EF-1β and could have a prominent role in the association of the β, γ and δ subunits [1,24]. The polypeptide domain involved in the association of the β and γ subunits has been mapped to the NH_2-terminal regions of the two proteins. This polypeptide segment corresponds to the region homologous to p18, shown in Fig. 2. Moreover, this homology domain is also recovered in the NH_2-terminal polypeptide extension of human valyl-tRNA synthetase, an enzyme that forms a stable complex with EF-1H [3,4]. It was shown that the removal of this extension by controlled proteolysis leads to a fully active enzyme that has lost its ability to associate with EF-1H [25]. Therefore, the conserved blocks shared by various components of the translational machinery are likely to correspond to a protein interface involved in their association.

The occurrence of a multisynthetase complex and of protein interactions between components of the translational machinery in higher eukaryotes could be the reflection of the in situ cellular organization of protein biosynthesis. Many weak and

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p18      60-LLGSTAEEKALVQQWLEYRITQV 12 LKDLNSYLEDKVYLAGYNITLADILLYYGL 14 YLNVSRWFCHIQH-151

EF1γHs   84-LRGSTPEAAAQVVQWVSFADSDI 32 LGLLDAYLKTRTFLVGERVTLADITVVCTL 15 FPNTNRWFLTCIN-196
EF1γOc   84-LRGSTPEAAAQVVQWVSFADSDI 32 LGLLDAHLKTRTFLVGERVTLADITVVCTL 15 FPNTNRWFLTCIN-196
EF1γAs   81-LRGSSDLEKAQIIQWMTFADTEI 32 LQALDDHLLTRTYLVGERITLADIVVTCTL 15 YVNTNRWFITLIN-193
EF1γXl   84-LRGTTRLHQAQVIQWVSFSDSHI 32 LGVLDSHLQTRTFLVGERITLADITVTCSL 15 FGNVTRWFVTCVN-196
EF1γEn   79-LLGTTGQEYASIIRWMAFGITEI 32 LKILDNHLCGREYLVGETLSLADLFVLGIV 15 HRNLSTWFERVHA-191
EF1γSp   82-LNGTTAEEKAKVLQYCSFTNSEL 32 FARFDEELASKTYLVGSRLTLADIFFTCFL 15 YTHIYRYYQTIYH-194
EF1γTc   86-LYGRTPLEGSQVDMWLDFSATEL 28 LRALEAWLETRTFLVGERMTVADVAVAFAL 17 YRNAYRMYNTVMQ-196

EPRSDm   69-LYGETAIERTQIDHWLSFSLTCE  6 LSFLTSPLPPVTYLVANKLTIADFALFNEM 12 PQHVQRWYDLITA-152
EPRSHs    1-       MEHTEIDHWLEFSATKL  9 INELNHCLSLRTYLVGNSLSLADLCVWATL 15 PVHVKRWFGFLEA-84
VRSHs    85-GGPGGSRAAVLVQQWVSYADTEL 31 LSPLEEWLRLHTYLAGEAPTLADLAAVTAL 15 WNNVTRWFVTCVR-196

GSTIo    81-LSGKTTEERATNTRWLSFFNSDF 26 LEYIDKHLSQSKYFTNNTILTADIFAFQIF 12 YPNVERFTGEVSQ-184
GSTDm    81-LLPNDPKKRAVINQRLYFDMGTL 29 FGFLDTFLEGQEYVAGDQLTVADIAILSTV 12 YSNVSRWYDNAKK-187

EF1βHs                              12-LQVLNDYLADKSYIEGYVPSQADVAVFEAV  7 LCHALRWYNHIKS-61
EF1βOc                              12-LQVLNDYLADKSYIEGYVPSQADVAVFEAV  7 LCHALRWYNHIKS-61
EF1βAs                              12-QEQLNELLANKSYLQGYEPSQEDVAAFNQL  7 FPYLLRWYKHISS-61
EF1βXl                              12-LKVLKEFLADKSYIEGYVPSQADVAVFDAL  7 LFHALRWYNHIKS-61
EF1βBm                              12-LNDLNQYLAEKSYVSGYTPSQADVQVFEQV  7 LPHVLRWYNQIAS-61
EF1βAt                              13-LKKLDEHLLTRSYITGYQASKDDITVFAAL  7 YVNASRWYNHIDA-62
EF1βOs                              14-LKKLDEYLLTRSYISGYQASNDDLAVYSAF  7 YTNVARWFTHIDA-63
EF1βTa                              14-LKALEAHLAGKTYISGDGITKDDVKVFAAV  7 FPNAARWYDTVAA-63
EF1βSp                              11-LKQLNDFLLDKSFIEGYEPSQADAVVFKAV  8 YPNGARWYKQIAT-61
EF1βSc                              12-LKQLNASLADKSYIEGTAVSQADVTVFKAF  3 YPEFSRWFNHIAS-57
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Fig. 2. Alignment of the amino acid sequences of p18 and related proteins. (Top) The sequence of the p18 protein from hamster is schematized with large segments indicating the three regions of homology with the γ (EF1γHs) and β (EF1βHs) subunits of elongation factor 1 from *Homo sapiens*, with the multifunctional glutamyl·prolyl-tRNA synthetase from *Drosophila melanogaster* (EPRSDm), and glutathione *S*-transferase from *Issatchenkia orientalis* (GsTIo). (Bottom) The actual alignment is only shown in the block region. The position of the first amino acid is indicated as well as the number of residues between the blocks. The origin of the sequences and their accession numbers are: EF-1γ from *H. sapiens* (Hs; P26641), *Oryctolagus cuniculus* (Oc; P29694), *Artemia salina* (As; P12261), *Xenopus laevis* (Xl; P26642), *Emericella nidulans* (En; U34740), *Schizosaccharomyces pombe* (Sp; P40921) and *Trypanosoma cruzi* (Tc; P34715); glutamyl·prolyl-tRNA synthetase from *D. melanogaster* (Dm; P28668) and *H. sapiens* (Hs; P07814); valyl-tRNA synthetase from *H. sapiens* (Hs; P26640); glutathione *S*-transferase from *I. orientalis* (Io; P30102) and *D. melanogaster* (Dm; D46681); EF-1β from *H. sapiens* (Hs; P24534), *O. cuniculus* (Oc; P34826), *A. salina* (As; P12262), *X. laevis* (Xl; P30151), *Bombyx mori* (Bm; P29522), *Arabidopsis thaliana* (At; P48006), *Oriza sativa* (Os; S41086), *Triticum aestivum* (Ta; P29546), *S. pombe* (Sp; D82574) and *Saccharomyces cerevisiae* (Sc; L22015).

dynamic interactions that do not survive cell disruption should also contribute to establish a cytoplasmic network of association of the various components of protein synthesis. Although no stable framework containing EF-1H and the multisynthetase complex could be isolated, a possible transient interaction between these two organized structures has been suggested [26]. Deutscher and co-workers [10,11] provided experimental support in favor of the channeling of tRNA in mammalian cells. In permeabilized cells, exogenously delivered aminoacylated or deacylated tRNA do not mix with endogenous tRNA, suggesting that the latter is channelled from one component of the biosynthetic machinery to another via a process of sequential and ordered protein-protein interactions.

In this connection, the sequence homology revealed in this study suggests that the p18 component of the multisynthetase complex could play a functional role similar to that of the polypeptide extension of valyl-tRNA synthetase, in anchoring the elongation factor EF-1H to the complex, in a transient manner, hereby facilitating the vectorial delivery of aminoacylated tRNA from the synthetase to the ribosome. The availability of the cloned cDNA, and the possibility to isolate large quantities of the recombinant protein, will be useful to clarify this issue.

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